

EVALUATION OF BIOACTIVE COMPOUNDS IN ETHANOL EXTRACT OF *Hylocereus undatus* FRUIT USING GCMS TECHNIQUE

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ABSTRACT

GC-MS is the best sensitive technique used for identification of the many structurally complex components that are present in plant extracts. *Hylocereus undatus* has been reported to have many medicinal properties and it is traditionally used. This study was implemented to actualize the chemical components of *Hylocereus undatus* fruit using Gas Chromatography–Mass Spectrometry, our results of GCMS compounds in the extract was relevant to the National Institute of Standards and Technology (NIST) library. Twenty compounds were identified. The prevailing compounds are 1,2-Benzenedicarboxylic acid, diethyl ester, Pentadecanoic acid, 1-Hexadecanol, 9-Octadecenoic acid, 9,12-Octadecadienoic acid, Stigmast-5-en-3-ol, (3 beta.,24S), Nonadecane and Tetratetracontane present in fruits of *Hylocereus undatus*. The results, it could be concluded that *Hylocereus undatus* may have antioxidant, anti-microbial, anti-cancer, anti-diabetic, hypocholesterolemic, and hepatoprotective activities due to the presence of secondary metabolites in the ethanolic extract.

Keywords: Gas chromatography and Mass spectroscopy, *Hylocereus undatus*, fruit extract,

INTRODUCTION

Plants are potent and powerful biochemists with a number of phytochemicals incorporated that prevent and treat several disorders. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Various medicinal plants have been used as a source of medicine for years in daily life to treat diseases all over the world (Kiruthika *et al.*, 2011). Traditional systems of medicines are prepared from a single plant species or combinations of several plant species. The bioactive component of the plant may be derived from any parts of the plant like leaves, roots, bark, flowers, fruits and seeds (Cragg and Newman, 2001).

Plants or plant products have been used as herbal medicines due to their curative properties since ancient times. Several bioactive compounds present in these plants constitute their therapeutic value (Leyla *et al.*, 2015). These secondary metabolites show many biological activities, for example, antioxidant activity, antimicrobial (Alqahtani *et al.*, 2019, Zeynep *et al.*, 2020) antitumor, anti-inflammatory and antiallergic (Pereira *et al.*, 2009). These phytochemicals are the bioactive principles having a unique and complex structure to treat various ailments. Screening of plants by chromatographic methods provides information on its pharmacological activities which help to select the plant of medicinal property (Juszczak *et al.*, 2019).

Gas chromatography-mass spectrometry (GC–MS) is the accurate technique employed for the detection of functional groups and identification of various bioactive therapeutic compounds that are present in medicinal plants (Satapute *et al.*, 2019; Fan *et al.*, 2018). The traditional medicinal plants are known to produce various compounds responsible for different bioactivities. The goals of the present study were to identify and characterize the bioactive compounds present in ethanol extract of *Hylocereus undatus* fruit, using GC-MS.

MATERIALS AND METHOD

Collection of plant materials

The fruits of *Hylocereus undatus* were collected from fruit shop, Thanjavur, Tamil Nadu, India. The collected fruits were washed in water, cleaned well to remove all traces of insects, dust and other kinds of impurities. The fruits were peeled, cut into pieces and collected the flesh and dried under shade for two weeks. The dried flesh was ground into powdered form and properly stored in sealed sterilized container for extraction and phytochemical analysis.

Preparation of extract

The dried flesh of dragon fruits (*Hylocereus undatus*) was extracted with ethanol using maceration technique for 24 h at room temperature. The extract was filtered by Whatman No. 42 (125mm) filter paper. The solvent was evaporated and concentrated under reduced pressure using rotary evaporator with the water bath at 45°C. The crude extract was further used for GCMS study.

GC –MS analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0.32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µl was employed (split ratio of 10:1) injector temperature 270 °C; ion-source temperature 200 °C. The oven temperature was programmed from 40 °C (isothermal for 2 min), with an increase of 8 °C/min, to 150°C, then 8°C/min to 250°C, ending with a 20min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 (Srinivasan *et al.*, 2013).

Identification of components

Interpretation on GCMS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Dr. Dukes, 2013).

RESULTS AND DISCUSSION

GC-MS is the best technique to identify the bioactive constituents such as long-chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino, and nitro compounds present in plant species. Hence, gas chromatography (GC) and mass spectroscopy (MS) associated with particular detection techniques have become sophisticated means for analysis of various compounds of medicinal importance (Velmurugan and Anand, 2017). The bioactive compounds from plants can be used as a chief molecule in the treatment of different ailments and help in drug invention. The current study was designed to identify the bioactive components using GCMS analysis. In the present study, twenty compounds were identified in the ethanol extract of *Hylocereus undatus* fruit by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 1 and Figure 1. The prevailing compounds are 1,2-Benzenedicarboxylic acid, diethyl ester, Pentadecanoic acid, 1-Hexadecanol, 9-Octadecenoic acid, 9,12-Octadecadienoic acid, Stigmast-5-en-3-ol, (3.β.,24S), Nonadecane and Tetratetracontane were found in this *Hylocereus undatus* fruit. The presence of various bioactive compounds justifies the use of the plant for various ailments by traditional practitioners. However isolation of individual

phytochemical constituents and subjecting its biological activity (Table 2) will definitely give fruitful results

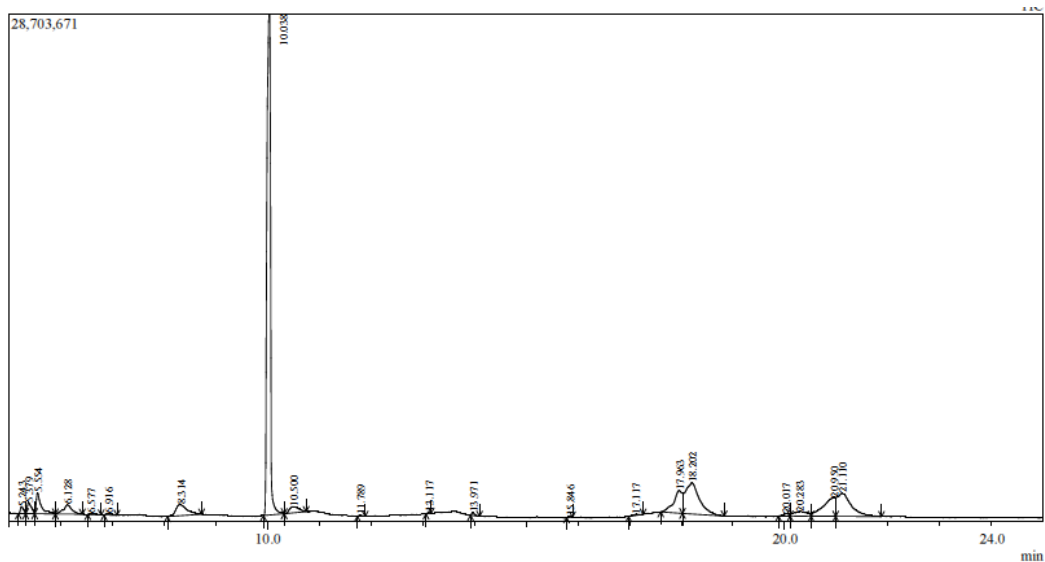


Figure 1: GC MS Chromatogram of *Hylocereus undatus* fruit

Table 1: Identification of active compounds in *Hylocereus undatus* fruit by GCMS analysis

Time	%	Int %	Molecular Formula	Molecular Weight	Name of the compounds
3			C ₇ H ₁₂ O		abicyclo[4.1.0]heptane, 1-methyl-
9			C ₇ H ₆ O ₃		hydroxymethyl)-2-furaldehyde
14			C ₁₀ H ₂₀ O ₄		-Propanetriol, 1-acetate
18			C ₁₆ H ₃₂ O ₁₂		ntanol, 2-methyl-, acetate
17			C ₁₂ H ₁₈ N ₂ O ₂		troso-2,4,4-trimethyloxazolidine
15			C ₁₆ H ₃₂ O ₂		noic acid, 3-methyl-, propyl ester
14			C ₁₂ H ₁₈ N ₄ O ₆		hosine
38	5	3	C ₁₄ H ₂₀ O ₄		benzenedicarboxylic acid, diethyl
10			C ₅ H ₆ D ₂ O ₂		02-trans-3,4-Dihydroxy-cyclopentene
39			C ₃₀ H ₆₀ O ₂		adecanoic acid
17			C ₃₄ H ₇₀ O		xadecanol
71			C ₄₆ H ₉₂ O ₂		tadecenoic acid
46			C ₃₂ H ₆₄ O ₂		Octadecadienoic acid
17			C ₁₇ H ₃₂ O ₂		est-5-en-3-ol (3.beta.)-, 9- lecenoate,
53			C ₅₀ H ₁₀₀ O		nast-5-en-3-ol, (3.beta.,24S)-
102	7		C ₄ H ₈ O ₃		droxy-4,4,6-trimethyl-7-

					tricyclo[4.1.0]heptan-2-one
17			140		decane
33			190		tetracontane
50			130 O ₂		,5a-Epoxy naphth[2,1-c]oxepin,
10			48O		A,6B,8A,11,11,14B-octamethyl-
	00	00			

Table 2: Biological activity of compounds identified in *Hylocereus undatus* fruit by GCMS analysis

Time	Name of the compounds	Biological activity**
38	Benzenedicarboxylic acid, methyl ester	microbial, Antifouling
39	Decanoic acid	microbial
47	Hexadecanol	microbial, Anti-oxidant , Anti-inflammatory.
71	Tridecenoic acid	hypertensive, Increase HDL and decrease Cholesterol
146	Octadecadienoic acid	anti-inflammatory, hypocholesterolemic cancer preventive, hepatoprotective , nematocide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge
53	Stigmast-5-en-3-ol, (3.beta.,24S)-	hepatotoxic , Antiviral, Antioxidant, Cancer preventive, Hypocholesterolemic
17	Decane	microbial, Antifungal.
33	Tetracontane	bacterial

**Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database].

Among the identified phytochemicals, 9,12-Octadecadienoic acid has been reported to exhibit anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocidal, insectifuge, antihistaminic, antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, and insectifuge properties. Stigmast-5-en-3-ol, (3.beta.,24S), an important bioactive compound of *Hylocereus undatus* was found to exhibit antihepatotoxic, antiviral, antioxidant, cancer preventive, and hypocholesterolemic properties, while 9-Octadecenoic acid has been reported to act as antihypertensive, increase HDL, and decrease cholesterol (Srivastava *et al.*, 2021; Mahmud *et al.*, 2021).

Findings from previous studies on GC-MS analyses of a vast array of plant extracts revealed that most of the herbal extracts with medicinal attributes contained some of the phytochemicals or analogs of the phytochemicals present in *Hylocereus undatus* extract. For instance, Octadecanoic acid from *indica* (neem) extracts (Pu *et al.*, 2010). Previous reports showed that 9,12-octadecadienoic acid (*Z,Z*)-, methyl ester from chloroform extract of *Albizia adianthifolia* (Schumacher) (Yu *et al.*, 2005; Abubakar and Majinda, 2016). Many of the compounds that have been identified in our study have

shown various types of biological activities. Various studies have already reported the antioxidant, anti-cancer and anti-inflammatory activities of these compounds (Sudha *et al.*, 2013; Aparna *et al.*, 2012; Hussein *et al.*, 2016).

Synergistic effects and additives phenomena are frequently vital to the bioactivity of different plant extracts but the activity of the purified compound is lost in some cases. It is supposed that crude organic, as well as purified aqueous fractions from the plants, are highly active biologically than the single isolated compound because of the synergistic effects. (Vural *et al.*, 2020; Usman *et al.*, 2020; Khaleed *et al.*, 2021).

CONCLUSION

The various bioactive compounds reported from the GC-MS analysis and subsequent literature evidences of their medicinal activities provide ample proof to the therapeutic and pharmacological potential of *Hylocereus undatus* fruit. Based on the results obtained in the present investigation, it may be concluded that the biological activities of the identified phytochemicals used for anti-microbial, anti-inflammatory, anti-diabetic, hepatoprotective, anti-hypercholesterolemic, and anti-cancer activities. Therefore, *Hylocereus undatus* fruit is recommended as a source of phytopharmaceutical value which needs to be further explored and validated so as to use it as a potential force in the field of health care against many diseases.

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